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Short communication

Interspecific variations in seed germination of *Corylopsis*

Mark S. Roh [a,*], Ae-Kyung Lee [b], Jeung Keun Suh [c], Carole M. Bordelon [d]

[a] US Department of Agriculture, Agricultural Research Service, US National Arboretum, Floral and Nursery Plants Research Unit, Beltsville, MD 20705, USA
[b] Sewon University, Department of Floral Art & Design, Cheongjiu 361-742, Republic of Korea
[c] Dankook University, School of Bio-Resources Science, Laboratory of Floriculture and Plant Physiology, Cheonan 330-714, Republic of Korea
[d] US Department of Agriculture, Agricultural Research Service, US National Arboretum, Gardens Unit, Washington, DC 20002, USA

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ABSTRACT

This study was initiated to investigate the differences in germination percentages and rates between *Corylopsis coreana* Uyeki and *Corylopsis sinensis* var. *calvescens* Rehder & E.H. Wilson following a warm stratification (WS) and cold stratification (CS), and to study the effect of different WS temperatures interacting with different durations of CS. Warm stratification at 10 °C, 15 °C, 20 °C, and 25 °C was given for 1 month (1 M 10 °C, 15 °C, 20 °C, and 25 °C WS) followed by 0 M, 1 M, 2 M, and 3 M of CS at 5 °C (0 M, 1 M, 2 M, 3 M CS) and seeds were germinated in an air conditioned greenhouse maintained at 18.5 °C/18 °C. On average, less than 1% of *C. coreana* seeds germinated when sown without any WS and CS or with 1 M 15 °C, 20 °C, and 25 °C WS without CS treatment. However, 26% *C. coreana* seeds germinated after 1 M 10 °C WS without any CS treatment. Germination was not affected by WS temperatures when followed by 2 M 5 °C CS. It is concluded that *C. coreana* exhibited low seed germination at 10 °C and that this temperature could be considered the upper limit of CS for *C. coreana*. Only 2 M CS was required for more than 90% seeds to germinate. However, *C. sinensis* var. *calvescens* required longer than 3 M CS for more than 29% seeds to germinate. This clearly shows that there is an interspecific variation in optimum dormancy-breaking requirements.

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1. Introduction

Seeds of many trees and shrubs require several months of warm stratification (WS) at approximately 20 °C followed by cold stratification (CS) at approximately 5 °C to break dormancy. Seeds can be prevented from germination due to a mechanical constraint imposed by the seed coat or a dormant embryo (Bewley, 1997). Warm and cold stratification could affect either the physical nature of a seed coat for efficient water absorption or the physiological nature of a dormant embryo, or both. After completion of proper WS and CS seeds can germinate when placed under favorable environment conditions. The optimum WS and CS treatments in various seeds have been reported (Dirr, 1990; Dirr and Heuser, 1987). However, the WS and CS treatments recommended for some seeds need to be refined. For example, only 1 month of WS (1 M WS) followed by 2 M CS was necessary for increased seed germination of *Styrax japonicus* Sieb. et. Zucc (Roh and Bentz, 2003; Roh et al., 2004) despite the suggestion of 3–5 M WS followed by 3 M CS for seeds to germinate (Dirr, 1988).

Corylopsis are shrubs or small trees, in the witch hazel family, Hamamelidaceae, and there are about 29 species native to China, Japan, and Korea with 19 species endemic to China (Zhang et al., 2003). The nature of dormancy in *Corylopsis* whether it is attributed to the seed coat or the embryo alone or a combination of the two constraints has not been investigated. Generally, it has been suggested that *Corylopsis glabrescens* seeds require 5M WS and 3 M CS (Dirr, 1990) for seeds to germinate. However, when *C. gotoana* Makino [*Corylopsis glabrescens* Franch. & Sav. forma *gotoana* (Makino) T. Yamanaka] (NA 67499H), *C. pauciflora* (NA 65653), *Corylopsis coreana*, *C. glabrescens* Franch. & Sab. (NA 50800H), and *C. spicata* Siebold & Zucc. (NA 36592) seeds were sown in the greenhouse maintained at 16–17 °C/14–15 °C (day/night) in October but decreased to 12–14 °C/8–10 °C in December, about 20% of the seeds germinated in about 1 month without any CS treatment, while other species, such as *Corylopsis sinensis* var. *calvescens* germinated only after a CS treatment (Roh, unpublished data). It is not known whether the interspecific variations of seed germination responses to WS and CS in *Corylopsis* are similar to interspecific or intraspecific variation in seed germination of *Chloris virgata* SW and *Dasyochloa pulchella* (Kunth) Willd. ex Rydb.

* Corresponding author at: USDA, ARS, US National Arboretum, Floral and Nursery Plants Research Unit, B-010A, Room 238, 10300 Baltimore Avenue, Beltsville, MD 20705, USA. Tel.: +1 301 504 5659; fax: +1 301 504 5096.
E-mail address: Mark.Roh@ars.usda.gov (M.S. Roh).

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Table 1
Effect of warm stratification (WS) and cold stratification (CS) on seed germination of *C. coreana* and *C. sinensis* var. *calvescens*

Temperature/Month[a]		*C. coreana*			*C. sinensis* var. *calvescens*		
WS	CS	Number of days to germination[b]		Final % of seeds germinated	Number of days to germination		Final % of seeds germinated[c]
		1st	Max		1st	Max	
0[d]	0	53	84	1	0	0	0
10	0	22	42	26	0	0	0
15	0	35	47	1	0	0	0
20	0	49	51	0	0	0	0
25	0	53	53	0	0	0	0
10	1	15	27	79	0	0	0
15	1	15	29	77	0	0	0
20	1	15	31	66	0	0	0
25	1	15	31	61	0	0	0
10	2	8	19	92	63	102	8
15	2	8	19	92	68	99	12
20	2	8	21	92	59	87	32
25	2	9	20	91	61	93	21
10	3	11	20	89	65	96	13
15	3	12	20	90	67	91	15
20	3	11	19	90	57	81	29
25	3	11	19	90	59	85	28
C. coreana					*C. sinensis* var. *calvescens*		
Regression analysis[e]					Level of significance, hsd 5%		
WS	Lin	**	*	**	8.3	10.6	8.1
	Quad	ns	ns	ns			
CS	Lin	**	**	ns			
	Quad	*	**	**			
WS × CS	Lin	**	*	*			

[a] Warm stratification (WS) temperatures (°C) and the number of month for cold stratification (CS).
[b] The number of days for first seed to germinate (1st) and to reach the maximum (Max).
[c] Average mean values of three replications.
[d] Seeds were sown without any warm (WS) and cold stratification (CS).
[e] Regression analysis was performed only with *C. coreana*. ns, *, **: non-significant, significant at 5%, 1%, *F*-test.

(Pezzani and Montaña, 2006), or to intraspecific variation in seed germination and survivorships in *Potentilla matsumurae* Th. Wolf (Shimono and Kudo, 2003), as well as in *Passiflora mollissima* Bailey and *P. tricuspis* Mast (Delanoy et al., 2006).

This experiment was, therefore, designed (1) to investigate the effect of WS temperatures and of duration of CS and (2) to investigate the differences of germination patterns of *C. coreana* and *C. sinensis* var. *calvescens* to document any interspecific variations in seed germination behavior responding to WS and CS temperatures.

2. Materials and methods

C. sinensis var. *calvescens* seeds (NA 57391) collected in October, 2003, from the US National Arboretum, Washington, DC, were stored dry at 5 °C in a refrigerator, and *C. coreana* [*Corylopsis gotoana* Makino var. *coreana* (Uyeki) T. Yamaz.] seeds collected in 2005 and stored dry at 5 °C were used in January, 2006. Seeds were soaked in tap water for 3 min to separate empty seeds that floated from full seeds that sank to the bottom of the beaker on January 31, 2006. Full seeds were collected, air-dried overnight at room temperature, and used for the germination test. Seeds were mixed with 125 cm^3 of Metro Mix 200 (W.R. Grace, Cambridge, MA) with 60% moisture content (weight), packed in polyethylene bags, sealed, and then treated at 10 °C, 15 °C, 20 °C, or 25 °C for 1 month of WS (1 M 10 °C, 15 °C, 20 °C, and 25 °C WS) in incubators and then treated for 0, 1, 2, and 3 months of CS (0 M, 1 M, 2 M, and 3 M CS) at 5 °C in a refrigerator starting from February 1, 2006. After treatments, seeds were sown in 15 cm diameter bulb pans filled with Metro Mix 200. One hundred seeds per replication and three replications per treatment were tested.

Seeds were germinated in 18.5 °C/18 °C, cooling/heating set point, air-conditioned greenhouse and pots were watered once a week or when needed to keep the medium moist. When cotyledons expanded, about 2 days following emergence of hooked hypocotyls through the surface of the medium, seeds were considered germinated (number of days to the first seed to germinate; Table 1), and counted twice a week or daily as needed, depending on germination rates as influenced by the duration of CS treatments. When no more seeds germinated over a 2-week period when data were collected twice a week, or over a week when data were collected daily after attaining the possible maximum germination (Max), data collection was terminated. Data was subjected to analysis of variance (ANOVA) and regression analysis was performed for *C. coreana* (SAS Institute, 1999). For *C. sinensis* var. *calvescens*, Tukey's ω-test [honestly significant difference (hsd)] at the 5% significance level is presented to compare means of treatment in which seeds had germinated. In this study, the number of seeds germinated is equal to the germination percentage.

3. Results

C. coreana seeds that did not receive any WS or CS started to germinate in 53 days after sowing, but only 1% of seeds germinated before data collection were terminated at 84 days (Table 1). When treated for 1 month of 10 °C WS and no CS (1 M 10 °C WS–0 M CS), 26% of seeds germinated in 42 days after sowing. However, when 1 month of WS temperatures was increased from 10 °C to 25 °C (1 M 15 °C, 20 °C, and 25 °C WS–0 M CS), less than 1% of seeds were germinated.

When treated with 1 M 10 °C WS–1 M CS, 79% of *C. coreana* seeds germinated in 27 days, the first seed germinated in 15 days. Regardless of having received 1 month of WS temperatures, seeds started to germinate in 15 days; however, maximum germination decreased from 79% to 61% as WS temperatures increased from 10 °C to 25 °C. When seeds received 1 M WS followed by 2 M or 3 M CS, seeds started to germinate in 8 and 11 days, respectively, and more than 90% of seeds germinated in 19–21 days after sowing. With 2 M or 3 M CS, germination was not affected by WS temperatures.

None of the *C. sinensis* var. *calvescens* seeds germinated when seeds were treated with no or 1 M CS. Seeds started to germinate in >59 days and >57 days after sowing when treated with 1 M WS and 2 M or 3 M of CS, respectively, and germination took over 87 days or 81 days, respectively (Table 1). The number of days for the first seeds germinated differed significantly between 2 M 15 °C WS–2 M CS and 3 M 20 °C WS–3 M CS. Maximum germination was less than 32% in all treatments, but WS temperatures affected seed germination of this species significantly.

4. Discussion

C. coreana seeds germinated after 10 °C WS without any CS treatment, while *C. sinensis* var. *calvescens* did not, exhibiting a species dependent germination pattern. The response of a limited number of seeds that can germinate without any CS reported here is referred to as "partial seed germination". Low seed germination observed in *C. coreana* (Table 1) was also observed in other species, such as *C. gotoana* (*C. glabrescens* f. *gotoana*) (unpublished data) and other species mentioned above in the introduction. However, this low and partial seed germination characteristic may not be able to distinguish these two and closely related taxa because all three, *C. coreana, C. gotoana*, and *C. glabrescens*, showed the partial seed germination pattern. A molecular study using markers generated by randomly amplified polymorphic DNA, however, revealed that *C. coreana* is different and distinct from *C. gotoana*, and *C. glabrescens*, and can be treated differently taxonomically from each other (Roh et al., 2007). Interspecific variation in seed germination was also reported in *Chloris virgata* Sw. and also in *Dasyochloa pulchella* (Kunth) Willd. Ex. Rydb. (Pezzani and Montaña, 2006), and based on the variation on germination requirements among *Sarraceana* species, observed differences in germination requirements of *S. purpurea* sp. *venosa* var. *burki*i Schell relative to other populations of *S. purpurea* was utilized to support the proposal to elevate this variety to a species status (Ellison, 2001).

The upper temperature limit of CS, or the lower temperature limit of WS could be between 14–15 °C/12–13 °C given in November and 12–14 °C/8–10 °C given in December, or accumulated temperatures that seeds were exposed during germination in the greenhouse. It is most probable that seeds may respond to 8–10 °C, which can be attributed to the partial germination response. Since temperatures higher than 10 °C in the experiment performed in an air-controlled greenhouse did not induce even low seed germination, the limit of CS could be 10 °C, which will induce some seeds to germination. Low temperature ranges that can induce seed germination for many woody plants were considered between 0 °C and 4.5 °C (U.S. Dept. Agric. Forest Service, 1974). At 14–15 °C/12–13 °C, water absorption would be facilitated, and dormancy could be broken at 12–14 °C/8–10 °C. Generally, temperatures ranging between 0 °C and 10 °C could be considered to break dormancy of seeds (Baskin and Baskin, 1998).

Fig. 1. Development of inflorescence of *C. coreana*. Flowers and seeds develop sequentially from the base of the inflorescence to the tip.

However, the observed partial seed germination could possibly result from a different level of seed dormancy, of a different physiological condition or an ineffective breaking of seed dormancy. This could be related to the position of seeds in the pendulous racemes, which mature at different times after anthesis (Fig. 1). It is not, however, known whether the level of dormancy in each seed collected from the same raceme may be at a different physiological condition. The different level of maturity of each individual seed based on the position at the inflorescence (intra-individual level) could result in differing levels of dormancy in *Corylopsis* and this could explain the observed partial seed germination. The position of seeds in the fruit affected seed germination in two populations of *Coincya rupestris* Porta & Rigo ex Rouy subsp. *rupestris*. Seeds from the indehiscent beak of the fruit of *C. rupestris* subsp. *leptocarpa* (Gonz.-Albo) Leadlay germinated at higher percentages than those from the valvar dehiscent portion (Herranz et al., 2003). However, this hypothesis cannot be accepted as a general trend (Copete et al., 2005).

To pinpoint the upper limit of CS for *Corylopsis* species that exhibited partial germination, more tests should be performed treating seeds at temperatures ranging from 5 °C to 15 °C for various durations of 0 M, 1 M, 2 M, and 3 M followed by 0 M, 1 M, and 2 M of CS at temperatures ranging from 5 °C to 10 °C. For more accurate determination of temperature, increments of 2.5 °C might be suggested to test in the future experiment. For species that do not exhibit a partial germination, longer than 3 M of CS should be given following 1 M WS as tested in this experiment. Germination percentage (>90%) could be considered maximum following the optimum WS and CS treatment for those species showing partial seed germination and the optimum duration of CS has been proven to be 2 months at 5 °C, regardless of seeds having received 1 month of WS temperatures.

Seed germination of *C. sinensis* var. *calvescens* might be different if fresh seeds are used for germination test. Perhaps, the level of dormancy in seeds stored in a refrigerator could be affected. Variation in the loss of seed dormancy during after-ripening of wild rice species from South America, *Oryza glumaepatula* and other species has been reported (Veasey et al., 2004). Germination of fresh harvested and dried stored seeds of *Styrax japonicus*, however, did not differ when the seeds received WS and CS to break dormancy (Roh and Bentz, 2003). Therefore, to understand whether seed germination will be affected by storage durations, further experiment should be performed with seeds that were harvested and stored in 5 °C refrigerator for 0, 2, and 4 years to see if there are any changes in dormancy level and germination percentage.

In conclusion, requirements of WS and CS for seed germination varied by species, which suggests that there is an interspecific variation in germination in the genus, *Corylopsis*. *C. coreana* seeds can germinate (about 26% of partial germination) without CS. *C. sinensis* var. *calvescens* failed to germinate to more than about 30% following 3 M CS at 5 °C. The upper limit of CS (or the lower limit of WS) is considered 10 °C for *C. coreana* and only 1 M WS and 2 M 5 °C CS were required for >90% seeds to germinate.

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